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HAZARDOUS WASTE SITE CHARACTERIZATION UTILIZING IN SITU AND LABORATORY BIOASSESSMENT METHODS.

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Abstract: Determination of adverse ecological effects at a hazardous waste site [HWS] requires definition of the questions to be assessed plus selection of appropriate measurement tools. Field observations conducted during the initial scoping activities play an important role in defining the ecological concerns to be addressed; the measurement tool box ideally consists of an array of direct field measurements [biological, chemical and physical], in situ bioassays, laboratory bioassays, additional analytical measures of site samples as well as statistical and risk assessment modeling. This paper discusses the assembly of the tool box and the selection of tools.

Introduction

Ecology is an integrative discipline which draws upon diverse sources of information [e.g. chemical, physical, geological, biological, etc.] to describe the interactions of organisms, populations, communities and ecosystems with each other and their surroundings. The completed ecological assessment of a hazardous waste site [HWS] should determine if an adverse ecological effect has occurred as a consequence of the materials present at the site (Norton et al. 1988). HWS assessments have historically evaluated human health effects [realized or potential]; chemical analysis of the site samples [soil, water, air]; and toxicity of site materials to selected bioassay organisms. Evaluations of toxicity and exposure have driven regulatory actions at HWS.

Hazard can be considered a function of exposure and toxicity; both toxicity and exposure may in effect be complex functions and be highly variable within problem-specific contexts. **Exposure assessment** may be regarded as a field activity, or an integrated lab/field task concerned with ecologically significant endpoints. For example, measurement endpoints may consider biological monitors [biochemical, physiological, or histological markers] or residue analyses of biological matrices and other environmental samples. **Toxicity assessment** is routinely regarded as being laboratory-derived; less commonly, toxicity assessment results from in situ methods that are completed within a field setting.

Relatively little effort has been directed toward ecological assessments. Whereas ecological assessments may draw upon chemical and toxicological data, neither chemistry nor toxicology should be construed as constituting an ecological assessment. Rather, it is necessary to define an ecological assessment endpoint in terms of a population inhabiting the site, a suite of populations, or an ecosystem process.

Approach to Ecological Assessment

Given budgetary restraints and time limitations, a great deal of care must be given to defining relevant assessment endpoints and selecting the appropriate measurements for a given site. From the outset, a considerable amount of information is available from which the options can be constructed; the

geographical [ecoregional] location and the probable chemicals can be defined and identified; and, case histories of similar hazardous wastes can be consulted. Recommended initial steps of the ecological assessment process are: assemble existing data sets including site maps, aerial photos, soils maps, geology and hydrology maps, and ecoregion maps; evaluate the appropriateness of an ecological assessment; and define the target zones to be examined.

The strategy for ecological evaluation incorporates varying levels of field sampling. The preliminary evaluation defines the ecological context of the site [ie., landscape features such as geomorphic, hydrologic, climatic, and biologic that potentially influence the site or define off-site transfer of toxicants and biota]; identifies the spatial extent of impact [current and potential] of the site and ecological features that warrant more detailed analysis for current assessment and/or future remediation monitoring.

During the past year, major accomplishments toward instituting ecological assessment into the Remedial Investigation/Feasibility Studies [RI/FS] activities were achieved. The Office of Waste Programs Enforcement and Office of Emergency and Remedial Response prepared a guidance document (US EPA 1989) to assist RPMs in instituting ecological assessments, and the Environmental Research Laboratory (ERL-C), Corvallis, Oregon, published the first guidance document on ecological site assessments methodologies (Warren-Hicks, et al. 1989). Much

remains to be accomplished.

One major point of concern arises from the fundamental misunderstanding of what constitutes an ecological assessment. The key word is integration. A significant obstacle in conducting ecological assessments is the poor delineation of utility and limitations of various tools available to assess site condition (Figure 1). Here we outline the capabilities and limitations of three components for evaluating measurement endpoints. These components of an ecological assessment are: 1) field surveys which focus on distribution and abundance of organisms [usually distinguished by taxonomic groups]; 2) bioassays designed to measure toxicity directly in the field or in the laboratory; and 3) biomarkers selected to report exposure to a specific chemical or class of chemicals.

1. Field Surveys

Assessment of ecological effects requires some measurement of structure and functional relationships of biota. The field component of an ecological assessment may be constructed to incorporate a variety of methodologies. Classical sampling designs and protocols for determination of populations of plants, animals, and microbes have been the subject of ecology from the inception of the discipline. Although no rigid guidelines for sampling are accepted universally, the concepts of adequacy of sample, objectivity, and precision are well entrenched in all field oriented studies. Researchers are given considerable

flexibility in modifying protocols to match the peculiarities of the site and the objectives of the sampling effort. Ecological sampling techniques, like all measurement activities, vary in rigor [ie., detail and/or accuracy] and in the effort [time and cost] required. Often, techniques that can be performed rapidly have inherent limitations of subsequent data manipulation and interpretation. However, rapid and low-cost procedures may provide the information desired. Guidance to plan ecological sampling should be derived from two leading questions "What do I need to know about the site?" [The Data Quality Objectives (DQO)] and "What do I plan to do with the information?" [Quality Assurance Work Plan (QAWP)]. Efficiency comes from matching the DQOs and QAWP.

Hazardous waste sites present unique restrictions of access and risk to workers. Because of extremely limited size and/or the nature of disturbance, some sites do not pose substantive ecological concerns. Proposed remediation actions may also minimize the level of effort that should go toward ecological evaluation. However, in a large number of sites, ecological assessment can play a major role in defining the nature of the problems associated with the site. Furthermore, ecological assessment should be considered a benchmark for evaluating the success of remedial actions in those situations where the nature of the site warrants action based upon a finding of adverse ecological impact.

Given the temporal limitations on data collection which

often pertain to hazardous waste sites, it is crucial to recognize the rather large error margins accompanying most of the resulting data. One-time sampling efforts almost always underestimate species richness. Ephemeral populations are easily missed. Quantitative estimates from one-time sampling efforts are static and thus miss the dynamics of the site. Nevertheless, indispensable information can be acquired from field sampling, in some cases through rather cursory reconnaissance [See Table 1].

Vegetation structure and to some extent composition can be determined remotely using conventional aerial photography, infrared photography, or more sophisticated radiometric signal such as the Thematic Mapper [TM] sensors available in satellites or fixed-wing aircraft [and the new ABRIS sensors under development]. To some extent, [especially with conventional aerial photography], archived data can be used to generate a history of land use. Such gross analyses permit generalized glimpses of spatial and temporal changes at and surrounding an HWS which can be informative not only of the vegetational responses but also suggestive of habitat conditions important for animal populations. More importantly, the infrared photography and radiometric sensors show great promise for use in defining the spatial boundaries of impact at an HWS. Because the plant leaves are sophisticated light harvesting assemblages, toxicants like those at many HWSs can alter the spectral reflectance patterns. If this property proves reliable, it will become a major tool to help delineate the spatial distribution of

phytotoxic substances.

Conventional ecological surveys of vegetation and animal populations can be used to generate patterns of distribution and abundance of the respective taxonomic groups. In most cases, acquiring accurate measurements of population sizes is costly and involves excessive on-site time which might pose unacceptable risk to the persons gathering the data. HWS conditions impose rigid demands that the DQOs be specified precisely and that the QAWP be equally targeted. Furthermore, as discussed earlier, we seldom have the basis to evaluate the long-term consequence of a given change in population numbers, particularly in light of the differential susceptibility of genotypic variants to a specified toxicant. This is a limitation of the science; it should not be construed as a fatal limitation of field surveys.

2. Bioassays to Determine Toxicity

Bioassays are instruments which yield some defined measurement [Figure 2]. The "sensor" and in most cases the "meter" in the bioassay instrument package is an organism. In theory the organism detects a multitude of signals, processes those signals in some fashion which may or may not be understood, and reports a quantifiable unit of measure [eg. death, growth rate, or other specified biological metric]. In this regard, a bioassay should be considered as any other instrument; an analytical tool equivalent to a pH meter, a gas chromatograph, a spectrophotometer, etc. As spectrophotometers may be modified or

adapted to permit different types of analyses, so can bioassays. Each instrument operates with some level of precision and accuracy. Each has boundaries defining legitimate uses.

In a regulatory sense bioassays have been indispensable in determining the permissible levels of chemical release into the environment [See Table 2]. Just as the medical profession has used the white rat or the rhesus monkey as surrogates of humans, environmental biologists have used the fathead minnow as a surrogate for fresh water fishes, daphnids as surrogates of aquatic invertebrates, radish or lettuce as surrogates for terrestrial vascular plants, and some would hold that a single "most sensitive" test organism could serve as a surrogate for the ecosystem as a whole. In the absence of better information, surrogates provide exceedingly valuable "range finding" information. From human health experiences we know that white rat studies can lead to false negative as well as false positive findings. We should not be surprised to encounter similar "mistakes" in performance of bioassays.

The greatest use of bioassays has been to determine the toxicity of single chemicals in simplified medium under controlled environmental conditions. A prime consideration of the bioassay organism is the ease of culturing in the laboratory. Another critical attribute is uniformity or in a statistical sense, narrow variance. Together these three features [controlled environment, "domestication," and homogeneity] run counter to environmental conditions. More recently, bioassays have been

employed to evaluate toxicity of complex mixtures such as effluent from waste water discharge or soil elution. Here these instruments perform an analytical function not achievable by other means; namely the integration of organism response from simultaneous exposure to multiple differentially toxic agents.

Toxicity testing typically incorporates an array of bioassay organisms representative of different trophic levels and varied life forms within trophic levels. Additionally, tests have been developed to discriminate among short exposures [acute]; long exposures [chronic]; maximum effect [lethal]; and sub-lethal effects [eg. reduced growth, reduced reproductive rate]. Although these options permit selection of an "instrument" which better approximates the organisms of interest [eg. one species of fish being the surrogate of another species of fish; a worm for a worm; etc.], the laboratory versions of bioassays seldom can be made representative of the exposure conditions and the myriad of

environmental factors that come to bear on organisms in the field.

Cognizant of such serious limitations, we are continuing efforts toward developing a broader array of bioassay organisms and toward adapting existing bioassay procedures so that the tests may be performed in situ. Successful examples of in situ terrestrial bioassays to date include detecting and monitoring environmental contamination using honey bees and earthworm bioassays. In the near term, it will be necessary to utilize a combination of laboratory and in situ assays. This duplicity is needed in order to provide appropriate calibration of laboratory and in situ measurements.

3. Biomarkers to Determine Exposure

Biomarkers are measures of molecular and/or physiological features of organisms which reveal a sublethal [often subtle] response to some stressor. A given biomarker response may be ephemeral or sustained; it may be specifically linked to a chemical or it may be associated with a general class of stressors. The biomarker response in most cases is measured in an individual and provides evidence that the individual in question has experienced exposure to the stress. Although this discipline of environmental biology is in its infancy, excellent tools exist; some with clearly defined relationships between the measurement endpoint and the assessment endpoint [See Table 3].

Several key virtues of biomarkers are flexibility for use in the lab or in the field as well as on cultured ["domesticated"] or wild organisms. Biomarkers can be used wisely to aid in defining relationships between laboratory and in situ bioassays, as well as relationships between bioassay organisms and the larger array of wild organisms.

Although several limitations to the generalized use of biomarkers for HWS assessment exist [eg. technical uncertainties regarding the sensitivity, interference, general applicability across taxonomic lines], some have been used very effectively to demonstrate adverse effects on organisms due to contaminants. Selected examples to illustrate use of the biomarker tool kit include cholinesterase, mutation frequency in plants, karyotype analysis, flow cytometry to measure cellular DNA content, DNA unwinding, and analysis of genetic diversity of populations via measurement of allelic distributions of metabolic enzymes. In all likelihood as more studies are completed, and as new biomarkers are perfected for field measurements, the theoretical framework to linking biomarker measurements to ecological endpoints will come into sharper focus.

Summary:

Each approach [ie., field surveys, toxicity tests, and biomarkers] contains numerous methods to acquire data for site assessments. Given the restrictions imposed by time, access, and resources, the selection of methods must be compatible with the

specific site DQOs. The collection of methods may be envisioned as a tool box from which one may "extract" the correct tool for the specified task (Figure 3). At ERL-C we are striving to define the specifications of the tools appropriate to perform ecological assessments of HWSs.

References

Norton, S., M. McVey, J. Colt, J. Durda, R. Hegner. 1988. Review of ecological risk assessment methods. Office of Policy Planning and Evaluation. USEPA, Wash D.C. EPA/230-10-88-041, 91pp.

Murphy, T. A. and L. A. Kapustka. 1989. Capabilities and limitations of approaches to in situ ecological evaluation. In Proceedings of Symposium on In Situ Evaluation of biological hazards of environmental pollutants. Plenum Press, New York. In Press.

Warren-Hicks, W., and B. Parkhurst (eds.). 1989. Ecological assessments of hazardous waste sites: a field and laboratory reference document. EPA 600/3-89/013 U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, OR.

U.S. EPA. 1989. Risk assessment guidance for Superfund--Environmental evaluation manual. 540/1-89/001A. Office of Solid Waste and Emergency Response, Office of Emergency and Remedial

Figure 1. Relationship Among Component Features of Site Assessments. The "Site" is illustrated conceptually as the ellipse labelled ENVIRONMENTAL. The unique portion of the ellipse [the upper zone] portrays non-biological, non-ecological measurement and assessment endpoints performed outside the context of ecological purview. The ECOLOGICAL sphere overlaps and integrates portions of ENVIRONMENTAL assessments, extends beyond the "Site" and can encompass toxicological, human health, and biomarker endpoints.

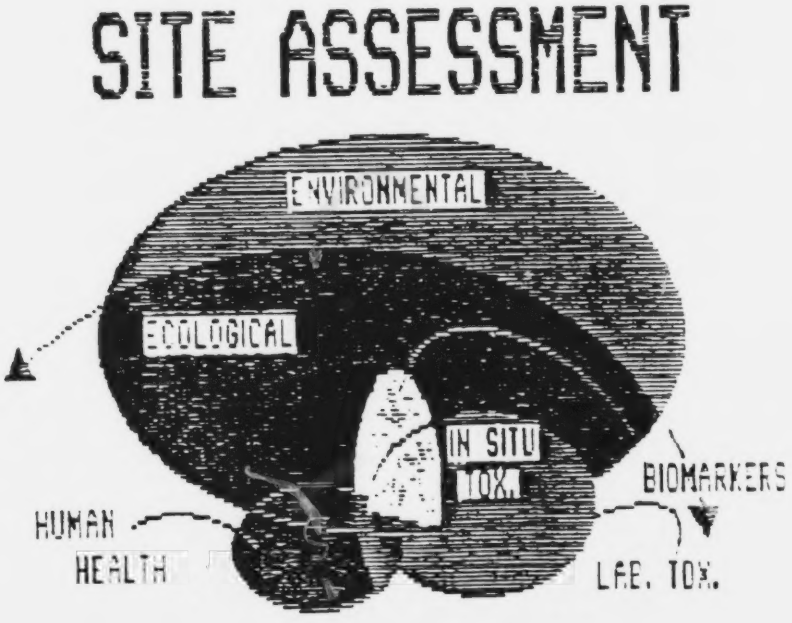

Figure 2. Conceptual model portraying common features of bioassays and a representative analytical instrument.

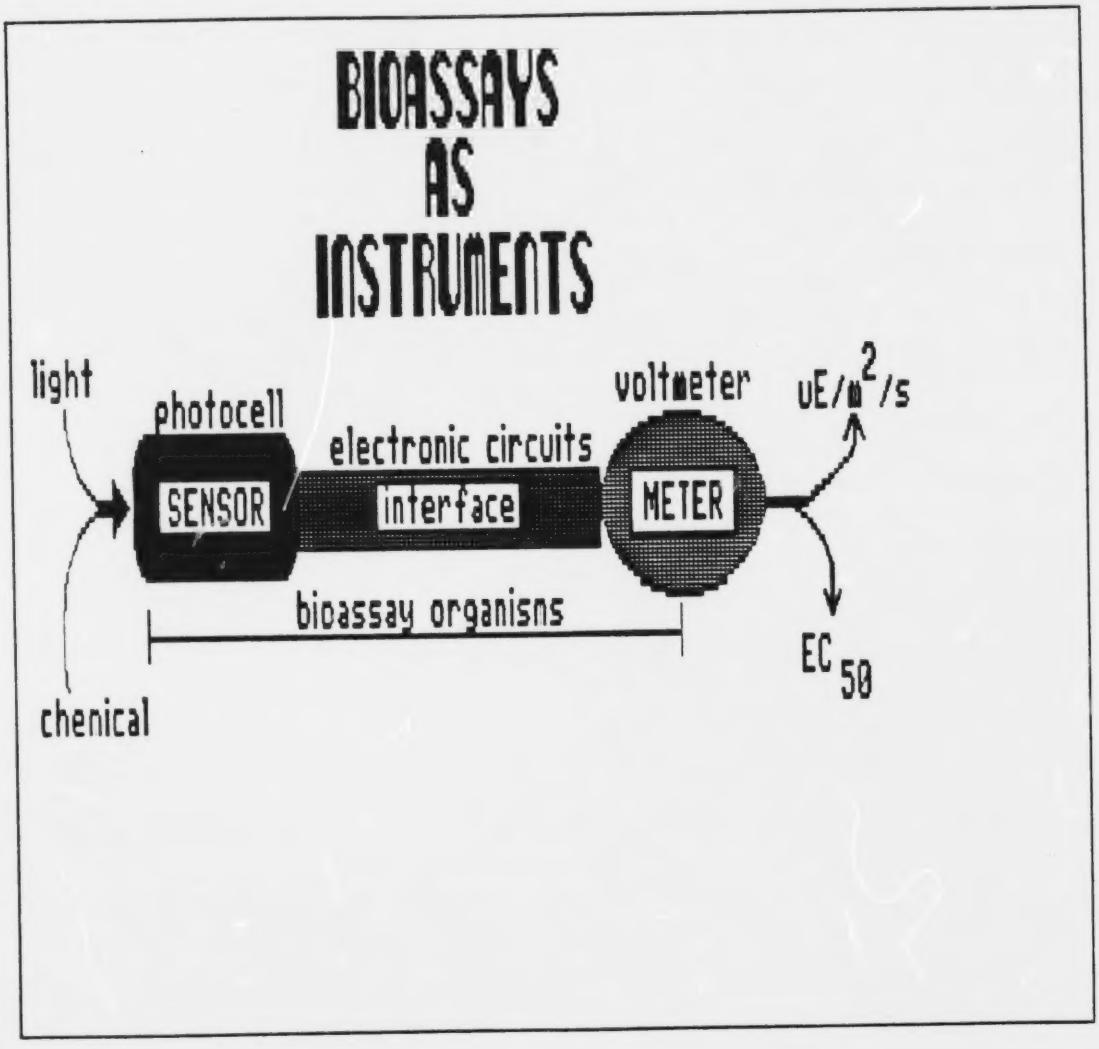

Figure 3. Bioassessment tool box for site assessments.

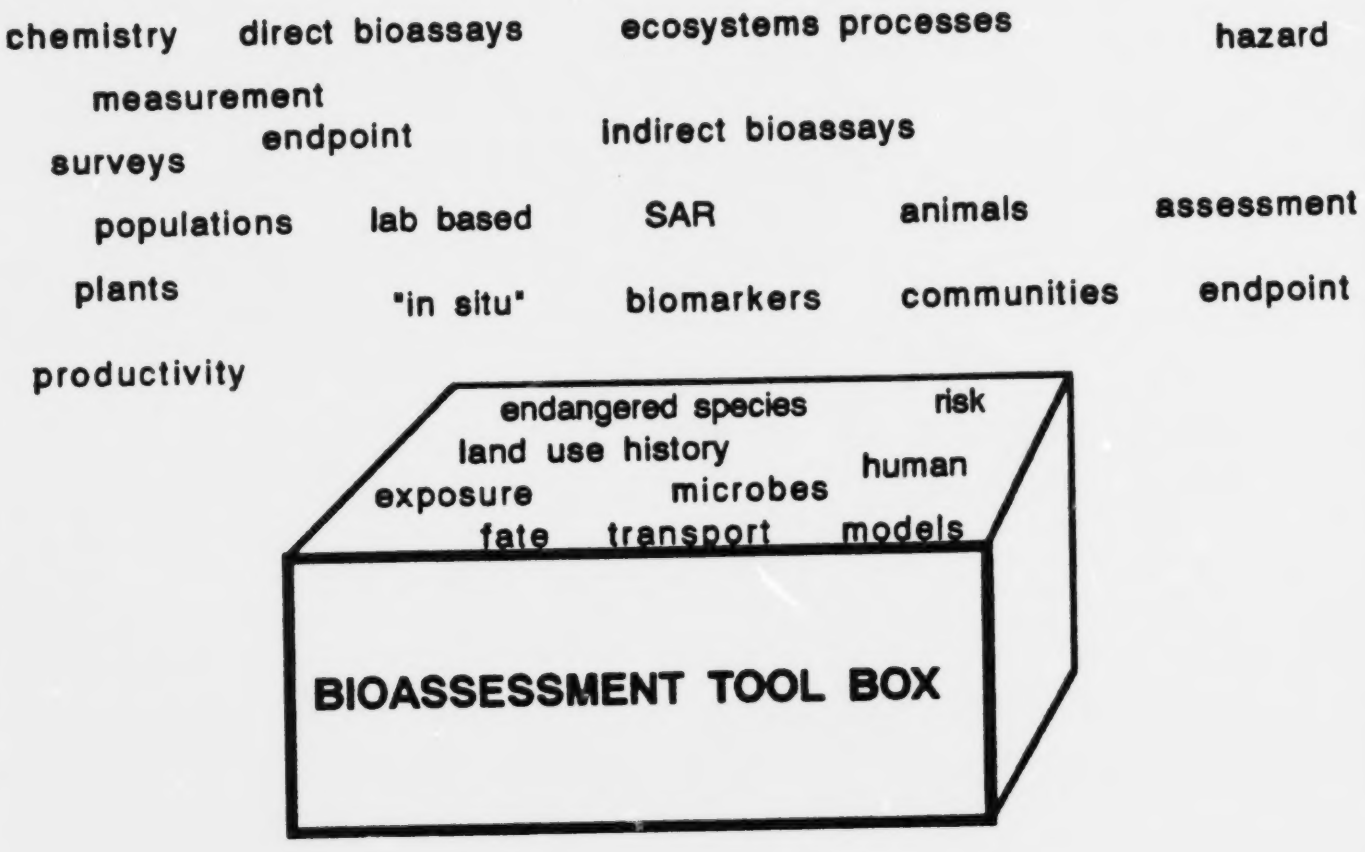

Table 1. Summary of capabilities and limitations of field surveys at HWSs (adapted from Murphy and Kapustka 1989).

Capabilities	
+	Surveys can be used to define endpoints of relevance.
+	A large selection of sampling techniques is available to permit desired measurement to a specified accuracy.
+	They are the most direct way to demonstrate adverse change.
+	Field surveys reflect the biological integration of all stresses.
+	Major vegetation components are amenable to sophisticated remote sensing technology.
Limitations	
-	Legal and safety concerns restrict site access.
-	Large natural variability may mask subtle but significant effects.
-	Detailed sampling can be expensive.
-	Survey data are restricted to correlative analysis.
-	Their "snapshot" view misses the dynamics [past and future].

Table 2. Summary of capabilities and limitations of toxicity tests for assessment of HWSs (Murphy and Kapustka 1989).

Capabilities

+ Tests can be used to establish causality.
+ They provide an extensive laboratory data base [especially from single chemical toxicity tests].
+ Multiple, simultaneous chemical stresses are integrated into a defined biological response.
+ The response "interprets" bioavailability.
+ Test conditions can be manipulated or adapted to meet different specification [including adaptation to *in situ* conditions].
+ There are many assays to choose.

Limitations

- Assay conditions [especially in the laboratory] are artificial.
- Tests are restricted to culturable organisms.
- Test organisms are selected to exhibit narrow statistical variance [ie., genetically diversity minimized]
- the artificial test conditions [especially in the laboratory] may not reflect proper exposure conditions.
- Extrapolation is restricted to individuals.

Table 3. Summary of capabilities and limitations of biomarkers for assessment of HWSs (Murphy and Kapustka).

Capabilities

+ Biomarkers provide evidence of exposure to sublethal concentrations of stressors.
+ They may be diagnostic.
+ They are amenable to both laboratory and field conditions.
+ This is a very active area of research showing great promise.

Limitations

- Linkage to ecological effects is not inherently clear.
- Only a few established biomarker systems are available.
- Use may be operationally complex.